Glycoconj J (2006) 23:453–460
DOI 10.1007/s10719-006-6979-6

Ectopic expression of *N*-acetylglucosamine 6-*O*-sulfotransferase 2 in chemotherapy-resistant ovarian adenocarcinomas

Akira Kanoh · Akira Seko · Hiroko Ideo · Midori Yoshida · Mitsuharu Nomoto · Suguru Yonezawa · Masaru Sakamoto · Reiji Kannagi · Katsuko Yamashita

Received: 14 October 2005 / Revised: 26 December 2005 / Accepted: 29 December 2005

Abstract Mucinous and clear cell adenocarcinomas are the major histological types of ovarian epithelial cancer and are associated with a poor prognosis due to their resistance to chemotherapy. A novel tumor marker specific for ovarian mucinous and clear cell adenocarcinomas would be helpful for overcoming these serious diseases. We showed previously by enzymological characterization and RT-PCR that colonic mucinous adenocarcinoma tissues ectopically express GlcNAc6ST-2, a member of the carbohydrate 6-*O*-sulfotransferase family (Seko, A. et al. (2002) *Glycobiology* **12**, 379–388). Here, we prepared a GlcNAc6ST-2-specific polyclonal antibody for immunohistochemical analysis and found that GlcNAc6ST-2 is ectopically expressed by not only colonic mucinous adenocarcinomas but also ovarian mucinous, clear cell and papillary serous adenocarcinomas. In contrast, solid serous adenocarcinomas, endometrioid adenocarcinomas, and mucinous adenomas expressed GlcNAc6ST-2 much less frequently or not at all. RT-PCR analysis confirmed that GlcNAc6ST-2 transcripts are expressed in ovarian mucinous adenocarcinomas but not in mucinous adenomas. In addition, immunohistochemical analysis using sulfated glycan-specific monoclonal antibodies showed that ovarian adenocarcinoma cells express GlcNAc 6-*O*-sulfated glycans, including an L-selectin ligand and its related glycans. These results indicate that GlcNAc6ST-2 would be a novel tumor antigen that is specifically expressed in ovarian mucinous, clear cell and papillary serous adenocarcinomas.

Keywords Ovarian cancer · Tumor marker · Sulfotransferase · Mucinous adenocarcinoma · Sulfated glycan

Abbreviations

Gal	galactose
GalNAc	*N*-acetylgalactosamine
GlcNAc	*N*-acetylglucosamine
HRP	horseradish peroxidase
LacNAc	*N*-acetyllactosamine

Introduction

Ovarian cancer is one of the most frequent causes of cancer-induced death in women and encompasses several common gynecological malignancies. Ovarian epithelial cancers can be clasified into histological types that also vary in chemosensitivity [1–3]. These include solid serous and endometrioid adenocarcinomas, which are sensitive to *cis*-platinum-based chemotherapy, and mucinous, clear cell and papillary

A. Kanoh
Hanno Discovery Center, TAIHCO Pharmaceutical Co. Ltd., Saitama, Japan

A. Seko · H. Ideo · K. Yamashita (✉)
Innovative Research Initiatives, Tokyo Institute of Technology, Yokohama; CREST, Japan Science and Technology Agency, Japan
e-mail: kyamashi@bio.titech.ac.jp
Fax: +81-45-921-4308

M. Yoshida
National Institute of Radiological Sciences, Chiba, Japan

M. Nomoto · S. Yonezawa
Department of Pathology, Kagoshima University, Kagoshima, Japan

M. Sakamoto
Department of Gynecology, Kyoundo Hospital, Tokyo, Japan

R. Kannagi
Department of Molecular Pathology, Aichi Cancer Center, Nagoya, Japan

serous adenocarcinomas, which are chemoresistant. A multivariate analysis showed that patients with solid serous and endometrioid adenocarcinomas have more favorable prognoses for survival than those with clear cell or mucinous adenocarcinomas [4]. Thus, an antigen specific for mucinous, clear cell or papillary serous adenocarcinomas would be helpful for early diagnosis of the disease, determining the prognosis, and monitoring the disease status. Moreover, a specific antigen could also serve as a novel target molecule in immunotherapy.

The levels of various tumor markers in cancer patient sera and tissues are often measured to determine the diagnosis and prognosis. CA125 is the most widely used marker of ovarian epithelial cancers and is considered to be the most useful marker at present [5,6]. However, CA125 has some limitations. In particular, the level of serum CA125 is not elevated in nearly half of all patients with stage I ovarian epithelial cancers [7]. Therefore, a novel tumor marker that is specific for the early stages of ovarian mucinous, clear cell, and papillary serous adenocarcinomas is highly desirable.

We previously demonstrated, using enzymological techniques and RT-PCR, that colonic mucinous adenocarcinomas ectopically express *N*-acetylglucosamine 6-*O*-sulfotransferase-2 (GlcNAc6ST-2) [8], and that non-mucinous adenocarcinomas and normal mucosa do not express this sulfotransferase. GlcNAc6ST-2 (HEC-GlcNAc6ST) belongs to the carbohydrate 6-*O*-sulfotransferase family, members of which transfer sulfate from adenosine 3′-phosphate 5′-phosphosulfate to the C-6 of Gal, GalNAc, or GlcNAc residues in various glycoproteins [9]. In normal human tissues, the expression of GlcNAc6ST-2 mRNA is limited to high endothelial cells of the lymph nodes, pancreas, and liver [10]. GlcNAc6ST-2 expressed in the high endothelial cells of lymphoid tissues is involved in the biosynthesis of the carbohydrate ligand for L-selectin, namely, GlcNAc-6-*O*-sulfated sialyl Lewis X [11,12]. The interaction of this ligand with L-selectin is required for the first step in the process of lymphocyte homing, namely, lymphocyte-endothelial cell adhesion. In colonic mucinous adenocarcinomas, the biological functions in which sulfated glycans synthesized by GlcNAc6ST-2 are involved remain unclear. However, we speculate that the sulfated glycans or the GlcNAc6ST-2 protein itself could serve as tumor markers of mucinous adenocarcinomas in general.

The ectopic expression of GlcNAc6ST-2 in colonic mucinous adenocarcinomas led us to examine whether this gene is expressed in mucinous carcinomas derived from other tissues. We were particularly interested in ovarian mucinous adenocarcinoma because of its poor prognosis. In this report, we used immunohistochemical and RT-PCR methods, and found that chemotherapy-resistant ovarian adenocarcinomas frequently express GlcNAc6ST-2, while benign mucinous adenoma cells and chemotherapy-sensitive ovarian adenocarcinomas express the protein with no or lower frequency. We also found that glycans related to L-selectin ligands and possibly synthesized by GlcNAc6ST-2 are distributed on the cell surface of mucinous, clear cell, and papillary serous adenocarcinoma cells.

Materials and methods

Materials

Fresh samples of normal colonic mucosa and differentiated and mucinous colonic adenocarcinomas were obtained from patients as described in our previous paper [8]. The study was approved by the Kagoshima University Faculty of Medicine Human Investigation Committee (No. H13-4).

Primary human ovarian tumor tissues were obtained, with informed consent, from 66 patients (ranging from 28 to 71 years of age) during surgical operations in Kyoundo hospital and Kagoshima University. The tumor tissues included 10 mucinous carcinomas, 14 clear cell carcinomas, 17 endometrioid carcinomas, 6 papillary serous carcinomas, 14 solid serous carcinomas, and 5 mucinous benign adenomas. Papillary serous carcinoma includes micropapillary architecture over 10% in carcinoma area. For immunohistochemical analysis (see below), the obtained tissues were fixed in 10% formaldehyde, dehydrated in a graded ethanol series, embedded in paraffin, and cut into serial sections using standard methods. For immunohistochemistry with mAb G72, frozen 10-μm thick sections were prepared from the tissues and fixed with acetone at -20°C for 20 min. For analysis of GlcNAc6ST-2 mRNA (see below), the tissues were immediately frozen in liquid nitrogen and later used for the isolation of mRNA.

Antibodies

A cDNA corresponding to the lumenal domain (amino acids 26-386) of GlcNAc6ST-2 was subcloned into the pBlueScript SK vector (Stratagene, La Jolla, CA) by PCR using the following primers: 5′-tttggatccATGTACAGCCACAACATC-3′ (forward) and 5′-tttaagCTTCTCAACCCTCTTAGT-3′ (reverse). The sequence was confirmed by using an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA). The amplified fragments were ligated into the pGEX-6p1 expression vector (Amersham, Piscataway, NJ) between its *Bam* HI and *Sal* I sites. *E. coli* strain BL21 was transformed with the GlcNAc6ST-2/pGEX-6p1 plasmid. Recombinant glutathione *S*-transferase (GST)-fused GlcNAc6ST-2 was produced and purified using Glutathione Sepharose 4B (Amersham) according to the manufacturer's instructions. An antibody against the truncated GlcNAc6ST-2 protein was then raised in rabbits according to standard procedures. The mouse monoclonal antibodies (mAbs) AG107, AG223, and

G72 (against 6-sulfo Lewis X, 6-sulfo *N*-acetyllactosamine, and 6-sulfo sialyl Lewis X, respectively) were prepared as described previously [13,14].

Preparation of soluble recombinant GlcNAc6ST-1, -2, -3, -4, and -5

The cDNAs encoding the lumenal domains of GlcNAc6ST-1 (amino acids 28-483), -2 (28-386), -3 (28-390), -4 (33-486), and -5 (27-395) [9] were prepared from the full length cDNAs generated previously [8] by polymerase chain reaction using gene-specific primers. The amplified cDNAs with the correct sequence were ligated into pQE-9-EK, which was generated by inserting the enterokinase cleavage site (DDDDK) into pQE-9 (QIAGEN) by using the QuikChange Site-Directed Mutagenesis Kit(STRATAGENE) and the oligonucleotide 5′-CCATCACCATCACGATGACGATGACAAAGGATCCG-TCGACC-3′ along with its complementary oligonucleotide. The *E. coli* M15 strain was then transformed with each of the resulting expression vectors. Recombinant soluble proteins were produced and purified using Ni-NTA agarose (QIAGEN) according to the manufacturer's instructions.

Immunohistochemical analysis

Immunohistochemistry was performed with the immunoperoxidase method using 3,3′-diaminobenzidine tetrahydrochloride (DAB, Wako Pure Chemical, Osaka, Japan). The paraffin-embedded tissue sections were deparaffinized in xylene and then rehydrated in a graded ethanol series. Endogenous peroxidases in all tissues were quenched with 0.3% hydrogen peroxide in methanol for 30 min at room temperature, after which the tissue sections were washed in 10 mM Tris-buffered saline (TBS, pH 7.4). The tissue sections were then blocked in 1% bovine serum albumin (BSA) in TBS for 1 h, followed by incubation overnight at 4°C with antibodies or normal rabbit serum diluted in TBS containing 0.1% BSA (1:500 dilution for the anti-GlcNAc6ST-2 antibody; 1:8 dilution for the AG107, AG223, and G72 antibodies; 1:500 dilution for normal rabbit serum). The sections were washed in TBS three times, incubated with biotinylated anti-mouse or anti-rabbit immunoglobulins for 10 min, washed, and then incubated with HRP-conjugated streptavidin for 15 min (DAKO LSAB2 System, DAKO Cytomation, Denmark). Finally, the sections were incubated with 0.1% DAB and 0.01% hydrogen peroxide in TBS for 10–15 min. After being rinsed in distilled water, the sections were counterstained with Mayer's hematoxylin, dehydrated, and mounted with Malinol (Muto Pure Chemical, Japan).

Detection of GlcNAc6ST-1, -2, - 3, -4 and -5 transcripts by RT-PCR

Total RNAs were isolated from ovarian tumor tissues by using the SV Total RNA Isolation System (Promega, Madison, WI) according to the manufacturer's instructions. cDNAs were synthesized from 1 μg of total RNA in a total volume of 20 μl of reaction mixture using SuperScript II reverse transcriptase (Invitrogen, Carlsbad, CA) and oligo(dT) primers. After cDNA synthesis, the reaction mixtures were diluted 5-fold with H_2O and 1–3 μl of each was used as a template for each PCR procedure. To normalize the mRNA quantities according to the β-actin mRNA levels, competitive PCR was performed using Gene Taq NT polymerase (Nippon Gene, Japan) in a total volume of 12 μl of reaction buffer containing 1 pg competitor plasmid DNA and 250 nM β-actin–specific primers. The competitor plasmid DNA was prepared previously [8]. PCR was performed under the following conditions: 25 cycles consisting of denaturation at 94°C for 30 sec, annealing at 65°C for 30 sec, and elongation at 72°C for 1 min. After PCR, an 8-μl aliquot was electrophoresed in a 0.9% agarose gel, followed by staining with ethidium bromide. The intensities of the amplified fragments were quantified by using an FLA-2000 multi-imager (Fuji Photo Film, Japan) and NIH Image software. The template volumes that yielded equivalent β-actin transcript levels were determined by acquiring the band intensity ratio of target and competitor DNAs; these volumes were used in the following procedures. PCR was performed with the determined template volume and the respective GlcNAc6ST-1, -2, -3, -4 and -5–specific primers [8], under the same conditions as for β-actin except that 33 cycles were performed.

Results

Immunohistochemical analysis of GlcNAc6ST-2 expression in ovarian mucinous adenocarcinoma tissues

We previously demonstrated that GlcNAc6ST-2 gene transcripts are ectopically expressed in colonic mucinous adenocarcinomas. To elucidate whether GlcNAc6ST-2 is also ectopically expressed in the mucin-producing carcinomas of various other organs, we raised a polyclonal antibody that recognizes the catalytic domain of GlcNAc6ST-2. To assess whether the polyclonal antibody specifically recognizes GlcNAc6ST-2 protein or not, we performed the Western blot analysis (Fig. 1). In SDS-PAGE analysis, recombinant GlcNAc6STs were detected at positions of the respective molecular sizes by SYPRO Orange staining. The polyclonal antibody recognized only GlcNAc6ST-2 protein, indicating the specificity of the antibody for GlcNAc6ST-2.

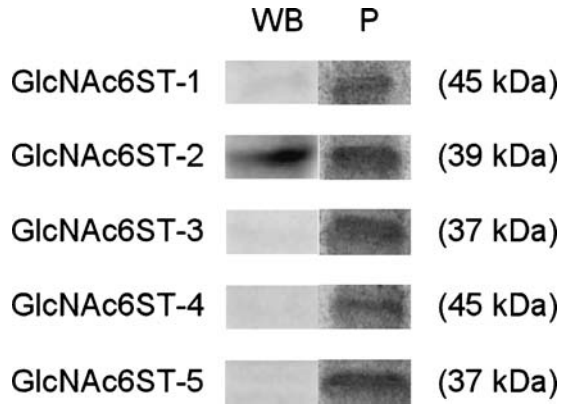

Fig. 1 Confirmation of specificity of anti-GlcNAc6ST-2 polyclonal antibody raised in this study by Western blot analysis using recombinant GlcNAc6STs. WB, Western blot analysis detected by anti-GlcNAc6ST-2 polyclonal antibody; P, Ponceau 3R stain of correspondent bands for five GlcNAc6ST isozymes.

To further confirm this result, we performed immunostaining of normal colonic mucosa and adenocarcinomas (Fig. 2). Our previous results [8,15] showed that GlcNAc6ST-2 is ectopically expressed in colonic mucinous adenocarcinomas, but not in normal coloninc mucosa and non-mucinous adenocarcinomas, using enzymological and RT-PCR methods. As shown in Fig. 2, normal colonic mucosa and non-mucinous adenocarcinomas were not immunostained by the polyclonal antibody, while mucinous adenocarcinomas were strongly immunostained, in good agreement with our previous results. Considering previous reports showing that GlcNAc6STs-1 and -3 are expressed in normal colonic mucosa [16,17], it is supported that the polyclonal antibody does not recognize at least GlcNAc6ST-1 and -3 in immunohistochemical staining. These results also support that the polyclonal anti-GlcNAc6ST-2 antibody is specific for the GlcNAc6ST-2 protein and can be applicable to immunohistochemical analyses.

We then used the anti-GlcNAc6ST-2 antibody to determine whether ovarian mucinous adenocarcinomas also express GlcNAc6ST-2. For this purpose, we tested 10 surgically obtained primary mucinous adenocarcinoma tissues. As shown in Fig. 3A and Table 1, all of the malignant ovarian mucinous adenocarcinoma tissues strongly expressed GlcNAc6ST-2, while none of the five benign mucinous adenoma cases we tested did so (Fig. 3B). GlcNAc6ST-2 was expressed in the mucin-producing cell layers in mucinous adenocarcinoma tissues, but not in interstitial cells.

RT-PCR analysis of GlcNAc6ST-2 transcripts in ovarian mucinous adenocarcinoma tissues

To confirm that GlcNAc6ST-2 is expressed in malignant ovarian mucinous adenocarcinomas but not in benign adenomas, we performed RT-PCR using RNA samples extracted from the surgically obtained tissues (three mucinous adenocarcinomas and two benign adenomas). To normalize the amounts of each cDNA, competitive PCR with β-actin cDNA was performed prior to PCR for detecting GlcNAc6ST-2 transcripts. The GlcNAc6ST-2 transcript was strongly or moderately expressed in the mucinous adenocarcinomas tested but not expressed at all in the mucinous adenomas (Fig. 4). In contrast, all mucinous adenoma and adenocarcinoma cases expressed GlcNAc6ST-1 transcripts while none expressed GlcNAc6ST-3, -4 or -5 transcripts (data not shown).

Immunohistochemical analysis of GlcNAc6ST-2 expression in other types of ovarian adenocarcinomas

To elucidate whether GlcNAc6ST-2 is expressed by other histological types of ovarian adenocarcinomas, we subjected surgically obtained primary serous, endometrioid, and clear cell adenocarcinoma samples to immunohistochemical analysis with the anti-GlcNAc6ST-2 polyclonal antibody. As shown in Table 1, 10 of 14 (71%) clear cell carcinomas and all six (100%) papillary serous carcinomas expressed GlcNAc6ST-2 at high frequency (Fig. 3C and D, respectively). Clear pools observed in clear cell adenocarcinomas were not stained while confined cytoplasms of the tumor cells were strongly stained. With respect to papillary serous

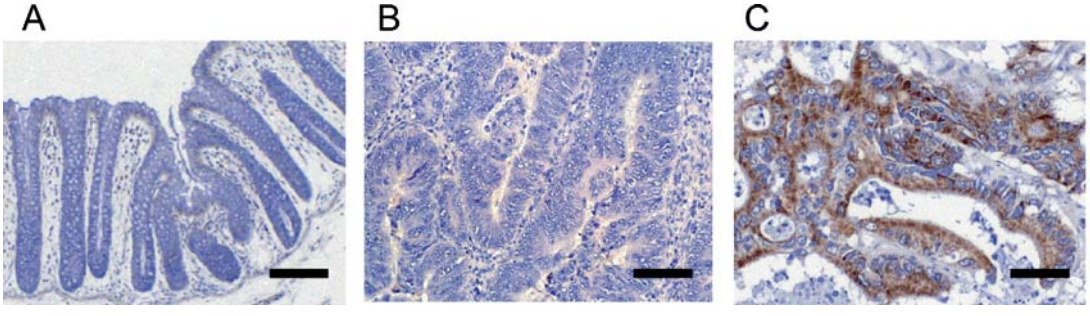

Fig. 2 Immunohistochemical staining for GlcNAc6ST-2 in colon tissue sections. A, normal colon mucosa; B, colonic differentiated adenocarcinoma; C, colonic mucinous adenocarcinoma. (Scale bars: A, 500 μm; B and C, 100 μm).

Table 1 Summary of immunohistochemical staining of GlcNAc6ST-2 in ovarian tumor tissue sections.

Histological classification	No. of cases with positive GlcNAc6ST-2 staining/total cases	Rate of GlcNAc6ST-2-positive staining
Mucinous adenoma	0/5	0%
Mucinous adenocarcinoma	10/10	100%
Clear cell adenocarcinoma	10/14	71%
Serous adenocarcinoma		
Papillary growth	6/6	100%
Solid growth	1/14	7%
Endometrioid adenocarcinoma	4/17	24%

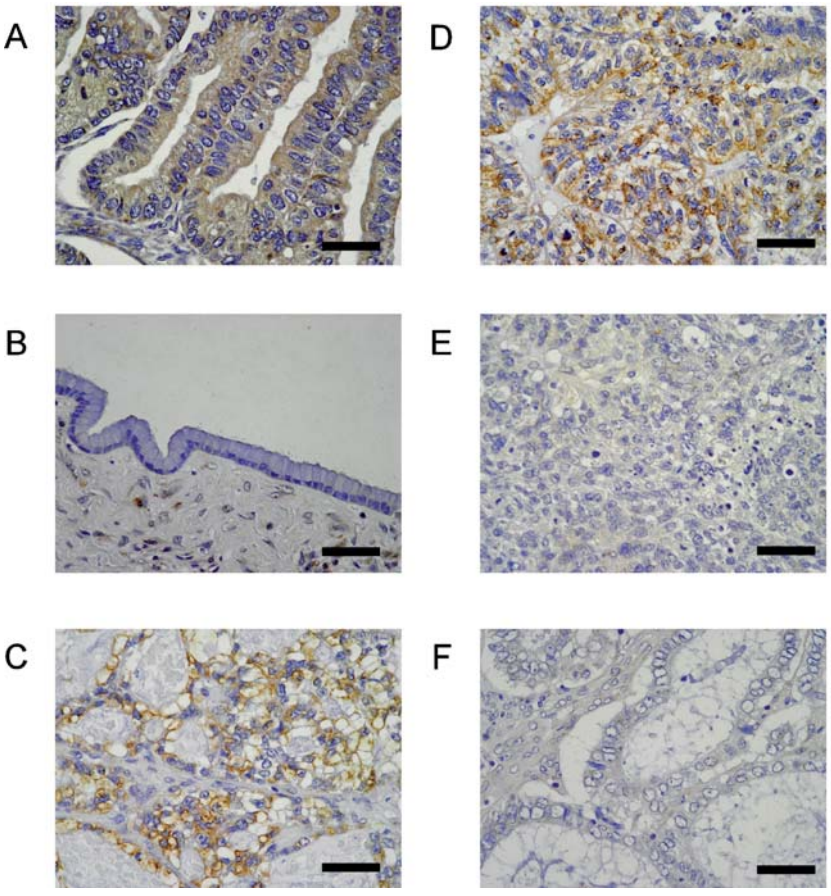

Fig. 3 Immunohistochemical staining for GlcNAc6ST-2 in ovarian tissue sections. A, mucinous adenocarcinoma; B, mucinous adenoma; C, clear cell adenocarcinoma; D, papillary serous adenocarcinoma; E, solid serous adenocarcinoma; F, endometrioid adenocarcinoma. (Scale bars, 50 μm).

carcinomas, most of the tumor cells expressed GlcNAc6ST-2. In contrast, only one of 14 (7%) solid serous carcinomas and four of 17 (24%) endometrioid carcinomas expressed GlcNAc6ST-2 (Fig. 3E and F, respectively). This indicates that the GlcNAc6ST-2 protein is expressed at quite different frequencies by ovarian cancer cells of diverse histological classifications. Thus, most or all of the ovarian mucinous, clear cell, and papillary serous adenocarcinomas ectopically expressed GlcNAc6ST-2.

It should be noted that chemotherapy-resistant types of ovarian adenocarcinomas exhibit frequent expression of GlcNAc6ST-2, while chemotherapy-sensitive types exhibit quite lower frequency of GlcNAc6ST-2 expression. It remains unclear whether or not there is molecular relationship between chemotherapy sensitivity and GlcNAc6ST-2 expression, but GlcNAc6ST-2 could be a tumor marker for chemotherapy-resistant types of ovarian adenocarcinomas as discussed later.

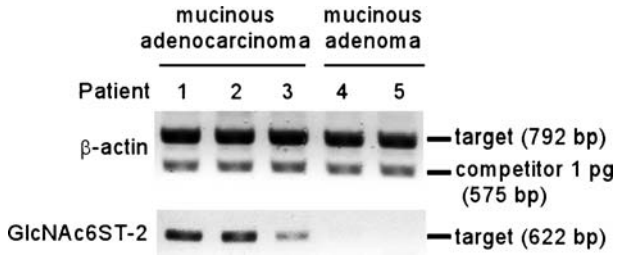

Fig. 4 Analysis of GlcNAc6ST-2 transcripts in mucinous adenocarcinomas and mucinous adenomas. Total RNAs were isolated from tissue blocks obtained from surgical operations and cDNAs were obtained by reverse transcription. The relative amount of cDNA of each sample was estimated by competitive PCR with 1 pg of β-actin–specific competitor DNA.

Detection of sulfated glycans in GlcNAc6ST-2-expressing ovarian adenocarcinomas by immunohistochemical analysis

To assess whether sulfated carbohydrate epitopes, which are the enzymatic products of GlcNAc6ST-2, are present in GlcNAc6ST-2-expressing ovarian adenocarcinoma tissues, we subjected one sample each of mucinous adenocarcinoma, mucinous adenoma, clear cell adenocarcinoma, papillary serous adenocarcinoma, solid serous adenocarcinoma, and endometrioid adenocarcinoma to immunohistochemical analyses using monoclonal antibodies specific for either 6-sulfo LacNAc (AG107), 6-sulfo Lewis X (AG223), or 6-sulfo sialyl Lewis X (G72). The three L-selectin ligand-related sulfated glycans examined are synthesized as follows. GlcNAc6ST-2 catalyzes the 6-*O*-sulfation of non-reducing terminal GlcNAc residues, followed by β1,4-galactosylation and α1,3-fucosylation of 6-sulfo GlcNAc, which generate 6-sulfo LacNAc and 6-sulfo Lewis X. The 6-sulfo sialyl Lewis X, an L-selectin ligand, is subsequently formed by the α2,3-sialylation and α1,3-fucosylation of 6-sulfo LacNAc. Thus, the 6-*O*-sulfation of GlcNAc by GlcNAc6ST-2 is the first step in the biosynthesis of all three sulfated glycans.

As shown in Fig. 5A and C, mucinous adenocarcinoma tissues expressed the 6-sulfo LacNAc and 6-sulfo Lewis X epitopes, as detected by mAb AG107 and mAb AG223, respectively. In contrast, the mucinous adenoma tissues did not express the carbohydrate epitopes recognized by mAb AG107 (Fig. 5B) or mAb AG223 (data not shown). The 6-sulfo sialyl Lewis X epitope detected by mAb G72 was also present in mucinous adenocarcinoma tissues (data not

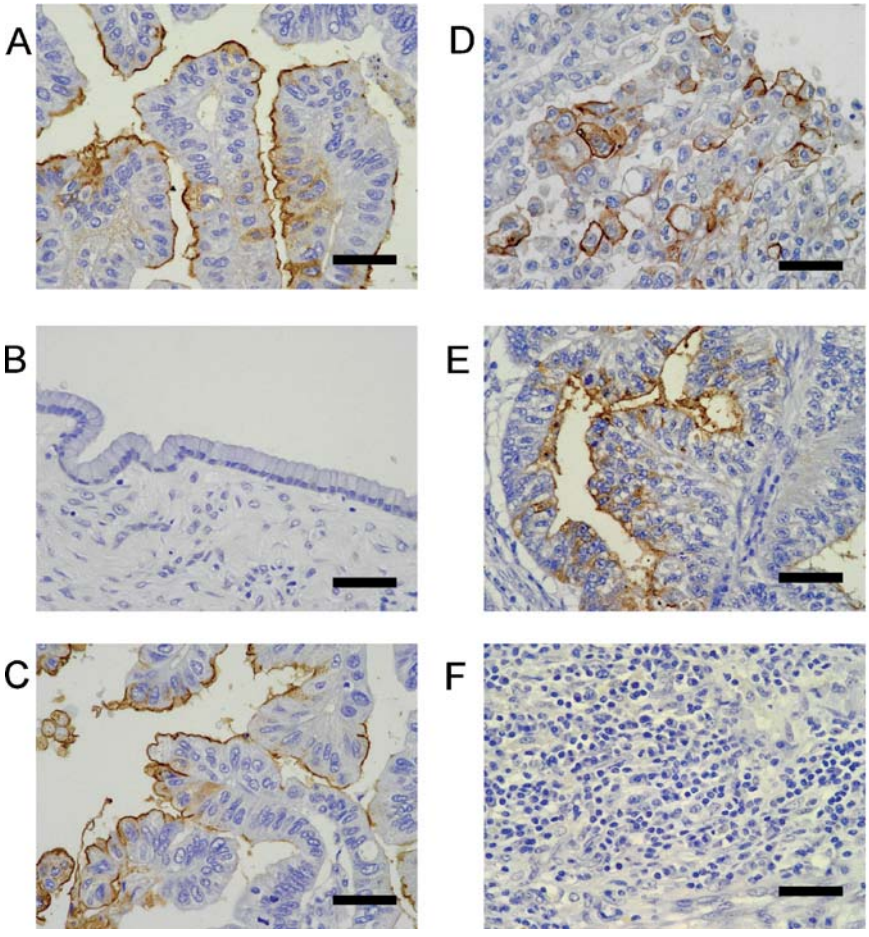

Fig. 5 Immunohistochemical staining with antibodies specific for 6-sulfo-LacNAc (AG107; A, B, D-F) or 6-sulfo-Lewis X (AG223; C). A and C, mucinous adenocarcinoma; B, mucinous adenoma; D, clear cell adenocarcinoma; E, papillary serous adenocarcinoma; F, solid serous adenocarcinoma. (Scale bars, 50 μm).

shown). In addition, the clear cell adenocarcinoma and papillary serous adenocarcinoma tissues expressed the carbohydrate epitopes recognized by mAb AG107 (Fig. 5D and E, respectively), while the solid serous and endometrioid adenocarcinomas did not (Fig. 5 and data not shown, respectively). These results suggest that the ectopic expression of GlcNAc6ST-2 by ovarian adenocarcinoma tissues correlates with their expression of 6-sulfo GlcNAc–containing epitopes on their cell surface membranes.

Discussion

We found in our previous study that GlcNAc6ST-2 transcripts and its enzymatic activities are present in colonic mucinous adenocarcinomas but not in non-mucinous adenocarcinomas or normal colonic mucosa [8]. Here, we examined the expression of GlcNAc6ST-2 by ovarian carcinomas as a first step to investigate whether GlcNAc6ST-2 is universally expressed by mucinous adenocarcinomas regardless of the organ of origin. We clearly demonstrated in this paper that ovarian mucinous adenocarcinoma tissues ectopically express GlcNAc6ST-2 transcripts, proteins and its carbohydrate products while mucinous benign adenomas do not. Furthermore, we showed that ovarian clear cell and papillary serous adenocarcinomas have a high incidence of GlcNAc6ST-2 expression, while solid serous adenocarcinomas and endometrioid adenocarcinomas both have a low incidence. Mucinous adenocarcinomas, clear cell adenocarcinomas and papillary serous adenocarcinomas account for over 50% of the total incidence of ovarian epithelial adenocarcinomas and are resistant to anticancer reagents like *cis*-platinum, resulting in a poor prognosis [1–3]. It remains unclear why these ovarian adenocarcinomas are resistant to these reagents, but one possibility is that the large quantity of sulfated mucins in mucinous adenocarcinomas and the accumulation of viscous materials in clear cell adenocarcinomas either might prevent the reagents from accessing their target molecules or otherwise interfere with their action. It would therefore be of considerable interest to determine whether the ectopic expression of GlcNAc6ST-2 in ovarian adenocarcinoma tissues is involved in their resistance to *cis*-platinum–based chemotherapy.

We also demonstrated that sulfated glycans, including L-selectin ligands, are expressed by ovarian mucinous adenocarcinomas. To our knowledge, this is the first report showing that ovarian mucinous adenocarcinomas express 6-sulfo-lactosamine-related antigens. Notably, Federici *et al.* have shown that ovarian mucinous adenocarcinomas express sialyl-Tn (sTn), Lewis a, and sialyl Lewis a antigens more frequently than serous and endometrioid adenocarcinomas [18]. In addition, Tamada and coworkers showed that cell lines derived from ovarian mucinous and clear cell adenocarcinomas express sulfated Lewis a, sialyl Lewis X and sialylated MUC1 antigen more strongly than those derived from serous adenocarcinomas [19]. Thus, it is thought that most mucinous adenocarcinomas have a tendency to express mucin-related carbohydrate antigens, including 6-sulfo-sialyl Lewis X antigen. Since it has been reported that glycans containing Lewis X–related structures are involved in the metastasis of cancer cells [20–23], we are thus interested in the potential role that the GlcNAc6ST-2-synthesized L-selectin ligands or related glycans play in ovarian adenocarcinoma metastasis or dissemination.

It remains unclear how GlcNAc6ST-2 transcripts are ectopically expressed in ovarian mucinous, clear cell and papillary serous adenocarcinomas. The ectopic expression suggests aberrant deregulation of GlcNAc6ST-2 gene transcription. The K-ras gene is known to be frequently mutated in colorectal and ovarian mucinous adenocarcinomas, whereas p53 is rarely mutated [24–26]. It has also been reported that serous adenocarcinomas showing micropapillary architecture commonly have a mutated K-ras gene and respond poorly to chemotherapy [3]. Interestingly, we here found that serous adenocarcinomas showing papillary architecture frequently express GlcNAc6ST-2. These observations together suggest that the ectopic expression of GlcNAc6ST-2 by ovarian adenocarcinomas, including papillary serous adenocarcinomas, may correlate with both their poor response to chemotherapy and the presence of K-ras gene mutations.

At present, CA125 is the most commonly used antigen for the serodiagnosis of ovarian cancers and its usefulness in this respect has been proven. However, only 50–60% of patients with stage I ovarian cancer are positive for the serum CA125 test [7]. Moreover, the substantial pseudo-positives associated with this test remain a problem for the early detection of ovarian cancer. Of the cases studied in this report, 70% of the patients with mucinous adenocarcinomas (60% of patients at stage I) and 40% of patients with mucinous adenomas had a CA125 serum level of over 30 units/ml. In addition, an immunohistochemical analysis showed that only 50% of mucinous adenocarcinomas express sTn (data not shown), another tumor marker for ovarian mucinous adenocarcinomas [27]. Thus, GlcNAc6ST-2, which is expressed in 100% of the mucinous adenocarcinomas and none of the mucinous adenomas, may be a better ovarian tumor marker than CA125 and sTn. Interestingly, in the clear cell adenocarcinomas cases, GlcNAc6ST-2 was expressed in all the Stage I cases (six out of 6) but in none of the Stage III cases (zero out of 3). This suggests that only the early stages of clear cell adenocarcinomas may express GlcNAc6ST-2, unlike mucinous adenocarcinomas, which seem to express GlcNAc6ST-2 regardless of the tumor stage (seven cases in stage I and three cases in stage III).

In this report, we demonstrated that GlcNAc6ST-2 is ectopically expressed by not only colonic mucinous

adenocarcinomas but also ovarian mucinous adenocarcinomas. Investigation of the mucinous carcinomas derived from other organs will be required to determine whether ectopic expression of GlcNAc6ST-2 is a common characteristic of all mucinous carcinomas. Nevertheless, our results indicate that GlcNAc6ST-2 may be a useful clinical marker for ovarian mucinous, papillary serous, and clear cell adenocarcinomas.

Acknowledgments Part of this work was supported by the Foundation for Promotion of Cancer Research, Japan.

References

1. Rubin, S.C., Wong, G.Y., Curtin, J.P., Barakat, R.R., Hakes, T.B., Hoskins, W.J.: Platinum-based chemotherapy of high-risk stage I epithelial ovarian cancer following comprehensive surgical staging. Obstet. Gynecol. **82**, 143–47 (1993)
2. Hogberg, T., Carstensen, J., Simonsen, E.: Treatment results and prognostic factors in a population-based study of epithelial ovarian cancer. Gynecol. Oncol. **48**, 38–49 (1993)
3. Shih, IeM., Kurman, R.J.: Ovarian tumorigenesis: A proposed model based on morphological and molecular genetic analysis. Am. J. Pathol. **164**, 1511–18 (2004)
4. Omura, G.A., Brady, M.F., Homesley, H.D., Yordan, E., Major, F.J., Buchsbaum, H.J., Park, R.C.: Long-term follow-up and prognostic factor analysis in advanced ovarian carcinoma: the Gynecologic Oncology Group experience. J. Clin. Oncol. **9**, 1138–50 (1991)
5. Jacobs, I.J., Skates, S., Davies, A.P., Woolas, R.P., Jeyerajah, A., Weidemann, P., Sibley, K., Oram, D.H.: Risk of diagnosis of ovarian cancer after raised serum CA 125 concentration: a prospective cohort study. BMJ **313**, 1355–58 (1996)
6. Jacobs, I.J., Skates, S.J., MacDonald, N., Menon, U., Rosenthal, A.N., Davies, A.P., Woolas, R., Jeyarajah, A.R., Sibley, K., Lowe, D.G., Oram, D.H.: Screening for ovarian cancer: a pilot randomised controlled trial. Lancet **353**, 1207–10 (1999)
7. Bast, R.C. Jr., Xu, F.J., Yu, Y.H., Barnhill, S., Zhang, Z., Mills, G.B.: CA 125: The past and the future. Int. J. Biol. Markers **13**, 179–87 (1998)
8. Seko, A., Nagata, K., Yonezawa, S., Yamashita, K.: Ectopic expression of a GlcNAc 6-*O*-sulfotransferase, GlcNAc6ST-2, in colonic mucinous adenocarcinoma. Glycobiology **12**, 379–88 (2002)
9. Fukuda, M., Hiraoka, N., Akama, T.O., Fukuda, M.N.: Carbohydrate-modifying sulfotransferases: structure, function, and pathophysiology. J. Biol. Chem. **276**, 47747–50 (2001)
10. Bistrup, A., Bhakta, S., Lee, J.K., Belov, Y.Y., Gunn, M.D., Zuo, F.R., Huang, C.C., Kannagi, R., Rosen, S.D., Hemmerich, S.: Sulfotransferases of two specificities function in the reconstitution of high endothelial cell ligands for L-selectin. J. Cell Biol. **145**, 899–910 (1999)
11. Hemmerich, S., Bistrup, A., Singer, M.S., van Zante, A., Lee, J.K., Tsay, D., Peters, M., Carminati, J.L., Brennan, T.J., Carver-Moore, K., Leviten M et al.: Sulfation of L-selectin ligands by an HEV-restricted sulfotransferase regulates lymphocyte homing to lymph nodes. Immunity **15**, 237–47 (2001)
12. Yeh, J.C., Hiraoka, N., Petryniak, B., Nakayama, J., Ellies, L.G., Rabuka, D., Hindsgaul, O., Marth, J.D., Lowe, J.B., Fukuda, M.: Novel sulfated lymphocyte homing receptors and their control by a Core1 extension beta 1,3-N-acetylglucosaminyltransferase. Cell **105**, 957–69 (2001)
13. Mitsuoka, C., Sawada-Kasugai, M., Ando-Furui, K., Izawa, M., Nakanishi, H., Nakamura, S., Ishida, H., Kiso, M., Kannagi, R.: Identification of a major carbohydrate capping group of the L-selectin ligand on high endothelial venules in human lymph nodes as 6-sulfo sialyl Lewis X. J. Biol. Chem. **273**, 11225–33 (1998)
14. Uchimura, K., Muramatsu, H., Kadomatsu, K., Fan, Q.W., Kurosawa, N., Mitsuoka, C., Kannagi, R., Habuchi, O., Muramatsu, T.: Molecular cloning and characterization of an N-acetylglucosamine-6-*O*-sulfotransferase. J. Biol. Chem. **273**, 22577–83 (1998)
15. Seko, A., Sumiya, J., Yonezawa,, S., Nagata, K., Yamashita, K.: Biochemical differences between two types of N-acetylglucosamine:-6sulfotransferases in human colonic adenocarcinomas and the adjacent normal mucosa: Specific expression of a GlcNac:-6sulfotransferase in mucinous adenocarcinoma. Glycobiology **10**, 919–29 (2000)
16. Lee, J.K., Bhakta, S., Rosen, S.D., Hemmerich, S.: Cloning and characterization of a mammalian *N*-acetylglucosamine-6-sulfotransferase that is highly restricted to intestinal tissue, Biochem. Biophys. Res. Coomun. **263**, 543–9 (1999)
17. Uchimura, K., El-Fasakhany, F.M., Hori, M., Hemmerich, S., Blink, S.E., Kansas, G.S., Kanamori, A., Kumamoto, K., Kannagi, R., Muramatsu, T.: Specificities of *N*-acetylglucosamine-6-*O*-sulfotransferases in relation to L-selectin ligand synthesis and tumor-associated enzyme expression. J. Biol. Chem. **277**, 3979–84 (2002)
18. Federici, M.F., Kudryashov, V., Saigo, P.E., Finstad, C.L., Lloyd, K.O.: Selection of carbohydrate antigens in human epithelial ovarian cancers as targets for immunotherapy: serous and mucinous tumors exhibit distinctive patterns of expression. Int. J. Cancer **81**, 193–98 (1999)
19. Tamada, Y., Iida, S., Aoki, D., Nozawa, S., Irimura, T.: Carbohydrate epitopes and mucins expressed by 17 human ovarian carcinoma cell lines. Oncol. Res. **11**, 233–41 (1999)
20. Phillips, M.L., Nudelman, E., Gaeta, F.C., Perez, M., Singhal, A.K., Hakomori, S., Paulson, J.C.: ELAM-1 mediates cell adhesion by recognition of a carbohydrate ligand, sialyl-Lex. Science **250**, 1130–32 (1990)
21. Takada, A., Ohmori, K., Yoneda, T., Tsuyuoka, K., Hasegawa, A., Kiso, M., Kannagi, R.: Contribution of carbohydrate antigens sialyl Lewis A and sialyl Lewis X to adhesion of human cancer cells to vascular endothelium. Cancer Res. **53**, 354–61 (1993)
22. Martin-Satue, M., Marrugat, R., Cancelas, J.A., Blanco, J.: Enhanced expression of alpha(1,3)-fucosyltransferase genes correlates with E-selectin-mediated adhesion and metastatic potential of human lung adenocarcinoma cells. Cancer Res. **58**, 1544–50 (1998)
23. Weston, B.W., Hiller, K.M., Mayben, J.P., Manousos, G.A., Bendt, K.M., Liu, R., Cusack, J.C. Jr.: Expression of human alpha(1,3)fucosyltransferase antisense sequences inhibits selectin-mediated adhesion and liver metastasis of colon carcinoma cells. Cancer Res. **59**, 2127–35 (1999)
24. Ichikawa, Y., Nishida, M., Suzuki, H., Yoshida, S., Tsunoda, H., Kubo, T., Uchida, K., Miwa, M.: Mutation of K-ras protooncogene is associated with histological subtypes in human mucinous ovarian tumors. Cancer Res. **54**, 33–35 (1994)
25. Zhang, H., Evertsson, S., Sun, X.: Clinicopathological and genetic characteristics of mucinous carcinomas in the colorectum. Int. J. Oncol. **14**, 1057–61 (1999)
26. Schuijer, M., Berns, E.M.: TP53 and ovarian cancer. Hum. Mutat. **21**, 285–91 (2003)
27. Inoue, M., Ton, S.M., Ogawa, H., Tanizawa, O.: Expression of Tn and sialyl-Tn antigens in tumor tissues of the ovary. Am. J. Clin. Pathol. **96**, 711–16 (1991)